U.S. FOOD & DRUG ADMINISTRATION

Human Foods Program

# Biotechnology Notification File No. 000202
# HFP Note to the File

**Date**: June 6, 2025

**From**: Matthew Fabian, Ph.D.

**To**: Administrative Record, BNF No. 000202

**Subject**: Cotton with insertion event MON 89151 (MON 89151 cotton)

**Keywords**: Cotton, *Gossypium hirsutum*, insect resistance, *Lepidoptera*, *cry1Da_7* gene, Cry1Da_7 protein, *cry1B.3* gene, Cry1B.3 protein, *vip3Cb1.1* gene, Vip3Cb1.1 protein, Bt, *Bacillus thuringiensis*, *Paenibacillus popilliae*, Bayer CropScience LP, MON 89151, OECD unique identifier MON-89151-3

## Summary

Bayer CropScience LP (Bayer) has completed a consultation with the Food and Drug Administration (FDA) on food derived from MON 89151 cotton. MON 89151 cotton expresses the Cry1Da_7, Cry1B.3, and Vip3Cb1.1 proteins, which confer enhanced resistance to various Lepidopteran insect pests. This document summarizes Bayer's conclusions and supporting data and information that FDA's Human Foods Program (HFP, we) evaluated pertaining to human food uses of MON 89151 cotton. FDA's Center for Veterinary Medicine summarizes its evaluation pertaining to animal food uses in a separate document.

Based on the safety and nutritional assessment Bayer has conducted, it is our understanding that Bayer concludes that:

- it has not introduced into human food a new protein or other substance that would require premarket approval as a food additive;
- human food from MON 89151 cotton is comparable to and as safe as human food from other varieties of cotton

HFP evaluated data and information supporting these conclusions and considered whether MON 89151 cotton raises other regulatory issues involving human food within FDA's authority under the Federal Food, Drug, and Cosmetic Act (FD&C Act). We have no further questions at this time about the safety, nutrition, and regulatory compliance of human food from MON 89151 cotton.

The U.S. Environmental Protection Agency (EPA) evaluates and authorizes the use of plant-incorporated protectants (PIPs) under the FD&C Act and the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). A PIP is defined in 40 CFR 174.3 as "a pesticidal substance that is intended to be produced and used in a living plant, or the produce thereof, and the genetic material necessary for the production of such a pesticidal substance," including "any inert ingredient contained in the plant, or produce thereof." In MON 89151 cotton, the Cry1Da_7, Cry1B.3, and Vip3Cb1.1 proteins constitute PIPs. Bayer states that, for all three proteins, pesticide registration applications and requests for tolerance exemptions have been filed

with EPA. The safety of the Cry1Da_7, Cry1B.3, and Vip3Cb1.1 proteins in MON 89151 cotton is under EPA's purview and is therefore not addressed in this document.

Bayer also communicated their intention to file a petition for the determination of nonregulated status with the U.S. Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) for MON 89151 cotton.

## Subject of the Consultation

| | |
|---|---|
| **Crop** | Cotton (*Gossypium hirsutum*) |
| **Designation** | MON 89151 |
| **Intended trait** | Insect resistance |
| **Developer** | Bayer CropScience LP |
| **Submission received** | March 4, 2025 |
| **Amendment(s) received** | None |
| **Intended use** | For general use in human food |
| **Transformation plasmid** | PV-GHIR529967 |
| **Expression cassette 1** | *cry1Da_7*, derived from *Bacillus thuringiensis* and conferring resistance to certain Lepidopteran insect pests |
| **Expression cassette 2** | *cry1B.3*, a chimeric gene derived from *Bacillus thuringiensis* and conferring resistance to certain Lepidopteran insect pests |
| **Expression cassette 3** | *vip3Cb1.1*, derived from a *Paenibacillus popilliae* and conferring resistance to certain Lepidopteran insect pests |
| **Method for conferring genetic change** | *Agrobacterium*-mediated transformation |

## Inheritance and stability

To generate MON 89151 cotton, Bayer transformed meristem tissues of cotton line DP393 using *Agrobacterium* to insert T-DNA region from plasmid PV-GHIR529967 into the cotton genome. The T-DNA region encodes expression cassettes for three insect resistance proteins: CryDa_7, modified from the *Bacillus thuringiensis* (Bt) Cry1Da; Cry1B.3, a chimeric protein derived from the Bt proteins Cry1Be and Cry1Ka2; and the *Paenibacillus popilliae* protein Vip3Cb1.1. Also encoded within the T-DNA region is a Cre-*lox* system in which two *loxP* recombination sites flank the selection marker gene *aadA*, conferring resistance to streptomycin and spectinomycin, and *cre*, encoding a Cre recombinase. Upon expression of *cre* in germline cells, Cre-*lox* recombination resulted in excision of the region of T-DNA with *cre* and *aadA* gene.

To verify the insertion event, Bayer utilized next-generation sequencing (NGS) and directed sequencing to assess insert copy number, the organization of the insertion, and the absence of plasmid backbone and excised T-DNA sequence in the genome. Using NGS reads from the R3 generation, Bayer identified the presence of a single insertion event for the region of T-DNA encoding the three insect resistance expression cassettes, with no insertion of plasmid backbone or other unintended DNA sequences (e.g.,

*cre* and *aadA*). This was confirmed via PCR and directed sequencing of the PCR products. Generational stability of the insertion event was assessed using NGS reads from five generations of MON 89151 cotton, which conveyed the presence of a single, identical insertion site in each generation. To evaluate the inheritance of the insert, the R2 generation was outcrossed with DP393 to yield the heterozygous R2F1, which was the basis for three generations of self-crossing. Individuals from those three generations (R2F2, R2F3, and R2F4) were surveyed for the presence of insert DNA via PCR, and the segregation ratios for the insert affirmed a Mendelian pattern of inheritance.

## Human Food Nutritional Assessment

The intended traits in MON 89151 cotton are not expected to alter levels of key nutrients or anti-nutrients. To assess potential unintended changes in composition relevant to safety or nutrition, Bayer analyzed samples of delinted seed from MON 89151 cotton and DP393, a non-genetically engineered non-(GE) control, grown in five U.S. planting sites in 2023. At each site, plants were grown in five randomized complete blocks, and samples were selected from each block and site. Seed samples were surveyed for analytes as detailed in the Organisation for Economic Co-operation and Development (OECD) Consensus Document for cotton,[1] including proximates, amino acids, fatty acids, carbohydrates (by calculation), fiber, vitamin E, calcium, phosphorus, and antinutrients (total gossypol, free gossypol, dihydrosterculic acid, and sterculic acid). Reference ranges for each analyte were sourced from published literature, including the Agriculture and Food Systems Institute Crop Composition Database.[2] Statistically significant (linear mixed model, $p < 0.05$) differences between MON 89151 cotton and non-GE control were identified for ten analytes (total protein, tryptophan, myristic acid, palmitic acid, palmitoleic acid, oleic acid, carbohydrates by calculation, malvalic acid, sterculic acid, and dihydrosterculic acid); however, all values reported fall within the range of values observed in the literature or the publicly available databases.[1, 2] Overall, Bayer concluded that food derived from MON 89151 cotton is compositionally comparable to that of non-GE control cotton with a history of safe use in food.

## Conclusion

Based on the information provided by Bayer and other information available to HFP, we have no further questions at this time about the safety, nutrition, and regulatory compliance of human food from MON 89151 cotton. We consider the consultation with Bayer on MON 89151 cotton to be complete.

Matthew Fabian -S

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[1] OECD (2009) Consensus document on compositional considerations for new varieties of cotton (*Gossypium hirsutum* and *Gossypium barbadense*): key food and feed nutrients and anti-nutrients. Series on the Safety of Novel Foods and Feeds No. 11. ENV/JM/MONO(2004)16 Organisation for Economic Co-operation and Development, Paris, https://one.oecd.org/document/env/jm/mono(2004)16/en/pdf

[2] AFSI (2024) Crop Composition Database. Agriculture & Food Systems Institute, https://www.cropcomposition.org